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A PHYSICO-CHEMICAL STUDY OF PECTIN

DISSERTATION

Submitted in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Pure Science, Columbia University in the City of New York.

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LUCIA E. EDSON, B.A., M.A.

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ACKNOWLEDGMENT

The writer wishes to express her sincere appreciation to Professor Arthur W. Thomas for suggesting the problem and for his kindly advice throughout the course of the work.

A PHYSICO-CHEMICAL STUDY OF PECTIN

INTRODUCTION.

Pectin is defined [1] as the water-soluble, methylated pectic substance occurring in plant tissue or obtained by restricted hydrolysis of protopectin. The term designates all intermediate substances between protopectin and pectic acid. The protopectin is the water-insoluble, unhydrolyzed, methylated pectic substances occurring in cell walls of plants and possibly in combination with cellulose. Pectic acid is water-insoluble and arises from the hydrolysis of pectin whereby methoxyl groups are eliminated.

Pectin is a colloidal carbohydrate derivative, differing from the polysaccharides by containing carboxyl groups. It differs from the mucilages in that the latter contain other acids than galacturonic acid.

Pectin possesses the property of forming jelly when added to a sugar solution at the right acidity. Neither protopectin nor pectic acid jellifies with sugar.

The structure of the pectin molecule is still under discussion.[2] In 1917 von Fellenberg[3] showed that pectin, on being treated with sodium hydroxide saponifies forming methyl alcohol and sodium pectate. He concluded that pectin is the methylated ester of pectic acid, not the calcium salt as a number of earlier investigators thought.

Ehrlich [4] obtained galacturonic acid from the products formed by hydrolyzing pectic acid with oxalic acid under pressure. By alkaline saponification of pectin, whereby methoxyl and galactose groups were split off, he isolated tetragalacturonic acid.

In 1916 Schryver and Haynes[5] determined the formula for pectic acid to be $C_{17}H_{24}O_{16}$. In 1922 Carre and Haynes[6] found that the precipitate formed by the addition of calcium

chloride after pectin had been saponified with sodium hydroxide and acidified with acetic acid gave the formula $C_{17}H_{22}O_{16}Ca$ and was therefore dibasic.

Nanji, Paton and Ling[7] isolated a substance which they considered as a fundamental unit of pectic substances. They determined the acid groups by heating the substance with hydrochloric acid and measuring quantitatively the carbon dioxide evolved. From the results the molecule must contain four galacturonic acid units, one arabinose unit and one galactose unit. They suggested that these units formed a hexa-ring, which on analysis gave the formula $C_{35}H_{50}O_{33}$. They considered pectin to be the corresponding ester in which the oxy radicles of the carboxyl groups are replaced by methoxyl groups.

Norris and Schryver[8] accepted this idea of a hexa-ring. They concluded three of the carboxyl groups in pectin to be methoxylated, while the fourth was free to act as an acid. Ahmann and Hooker[9] in 1926 claimed at least eleven carboxyl groups to the pectin molecule.

Pectin occurs in fruits, succulent edible vegetables and in cell wall tissue generally, and abundantly in such fruits as currants, grapes, and raspberries and in the albinos of lemons and oranges[10]. The data given here unless otherwise stated was obtained from apple pectin.

MATERIALS.

The apple pectin for this investigation was kindly supplied by the Douglas Pectin Company of Rochester, N. Y. Commercially the larger part of the pectin used in the household is said to be made by the Douglas process[11]. Apple peels and cores from canning factories and pomace from cider mills constitute the raw material. The sugars and flavor[12] are removed by leaching the press cake with water several times before extracting the pectin. In leaching, the press cake is kept covered with water several hours so that the sugars will diffuse away. The water is then drained off and the process repeated until the pulp is relatively free from

sugars. The press cake is then boiled to extract the pectin, steam passed in so that a temperature of practically 100° C is retained for a half to three quarters of an hour at atmospheric pressure. The solution contains about 0.3% of some acid like malic or citric.

This extract contains a small amount of protein but much starch [13] which has to be removed to prevent subsequent precipitation by tannin which is also present. The starch is therefore converted to dextrose or maltose by a diastatic enzyme and the solution is clarified by adding diatomaceous earth and filtering through cloth.

The apple pectin as supplied for this investigation had been once precipitated with alcohol. It contained about 7.2% ash.

In one part of the work, citrous pectin, furnished by the California Fruit Growers Exchange, was used for a comparison with the apple pectin. In the preparation of this pectin[14] the lemon pulp was soaked to remove the citric acid. The pectin was then extracted with hot 0.5% sulphurous acid solution. Much of the sulphur dioxide was driven off when the solution was cooled. Ammonia and aluminum sulphate were used to precipitate the pectin. The excess salts were washed out with water. The dried pectin was ground and washed with alcohol containing hydrochloric acid to remove aluminum salts and the acid removed by neutral alcohol. It was found in an analysis for this work that the pectin contained 7.01% water and 1.67% ash.

PURIFICATION.

For this investigation the apple pectin was further purified by the author using the usual method—which depends upon the solubility of pectin in water and its insolubility in alcohol. About two grams of pectin powder was dissolved in 100 cc of water and this solution was slowly stirred into two volumes of alcohol of at least 85 per cent, just acid with hydrochloric acid. The jellied mass was drained off through linen cloth and pressed in a cider press. The ash when this

process was twice repeated (making three alcohol precipitations) was reduced from 7.2% to about 2.2%.

The material was further purified by electrodialysis. The wooden apparatus used was the same as used by Murray[15] in his study of gum arabic. The box consisted of three compartments, each having a capacity of about 1,500 cc., the compartments being held together by bolts. The cells were coated with a water and acid proof paint. The pectin solution was poured into the central compartment and was separated from the distilled water in the outer compartments by pyroxylin coated linen which was mounted on wooden frames. A 240 volt direct current was used, sheet carbon electrodes, 8 x 12 cm serving as electrodes. The carbon anode disintegrated somewhat but the particles did not pass through the membrane.

Hydrochloric acid was added to the pectin solution at intervals of a few days to maintain an acidity of 0.01 molar. The pectin solution was mechanically stirred while the water in the outer compartments was continually changed so that the temperature was kept below 40° C. At the end of three weeks' electrodialysis, the ash was reduced to 0.18%.

Three separate batches of pectin were purified for this work. Each was electrodialyzed until the ash was 0.18% and until free from the chloride ion. The batches gave corresponding results in their properties even after standing in solution a few weeks.

PROPERTIES.

The pectin precipitated once with alcohol showed reducing sugar since one gram of the pectin gave a precipitate of six milligrams of cuprous oxide, following the modified Fehling method of Quisumbing and Thomas, but the pectin purified as described gave no precipitate of cuprous oxide.

A solution of the purified pectin containing one gram in 100 cc. of solution showed a pH value of 2.67, while unpurified pectin showed a higher pH value due to the ash content. The high hydrogen ion concentration cannot be attributed to the presence of a strong acid like hydrochloric or sulphuric as

the purified pectin gave no test for chloride ion nor for sulphur. Nor was there a test for phosphorus although the pectin before purification showed a phosphorus content of 0.61% and a 0.42% sulphur content. Nitrogen was likewise absent as shown by the Kjeldahl method. Despite its colloidal nature, pectin is, as Murray found ashless gum arabic, a fairly strong acid.

A summary of analyses made upon the pectin as received (once precipitated with alcohol), and upon the pectin purified as described before is given in Table I.

TABLE I.

	Pectin as received	Pectin (purified as described)
Ash (%)	7.20	0.18
Reducing sugar (mg. Cu_2O per 1 gm. pectin)	6.0	None
Dextrin	..	None
Chloride	..	None
Phosphorus (%)	0.61	None
Sulphur (%)	0.41	None
*$[a]_D^{25°}$	..	+173

Purified pectin when dried will redissolve in water but not easily. The pectin for this work was therefore kept in solution. The concentration in grams per cubic centimeters was determined by evaporating 50 cc. portions and drying at 45° C. in a vaccuum oven.

* This reading was obtained upon a 0.25% solution in a two decimeter tube in a Schmidt and Haensch quartz wedge saccharimeter, using white light filtered through bichromate solution. The Ventzke scale reading was converted to circular degrees by mutiplying by 0.3466.

TITRATION.

Electrometric titrations were made* using the bases, sodium hydroxide and barium hydroxide and the acids, hydrochloric and phosphoric. These are shown in Figure 1 and the data is given in Table II. The number of cubic centimeters of tenth normal acid or alkali are plotted as abscissae against pH as ordinates. The points for the two bases lie on the same curve, showing that pectin combines with alkali in equivalent quantities. The fact that the change in concentration of hydrogen ions in the vicinity of the stoichiometric point is fairly abrupt, shows pectin to have properties of a strong acid. The change in the concentration of hydrogen ions is very rapid from pH 5 to pH 9. with the addition of little alkali. The stoichiometric point at pH 7 indicates that 11.6 cc. of tenth normal base unites with one gram of pectin or the equivalent weight of pectin according to this method is 860. The curves with hydrochloric and phosphoric acids would indicate that pectin does not combine with acids and therefore is not amphoteric. This again resembles the property of ashless gum arabic.

Data for the titrations of sodium hydroxide and hydrochloric acid with citrus pectin, previously described, are given in Table III and the curves in Figure 1. The titrations were made on the material as received without further purification. The pH of a solution containing one gram dry weight in 100 cc. of solution was 2.65, practically the same as that of the purified apple pectin. The same type of curve was obtained in titrating with the base and acid as resulted from the apple pectin except that the change in the concentration of hydrogen ions in the vicinity of the stoichiometric point is not as

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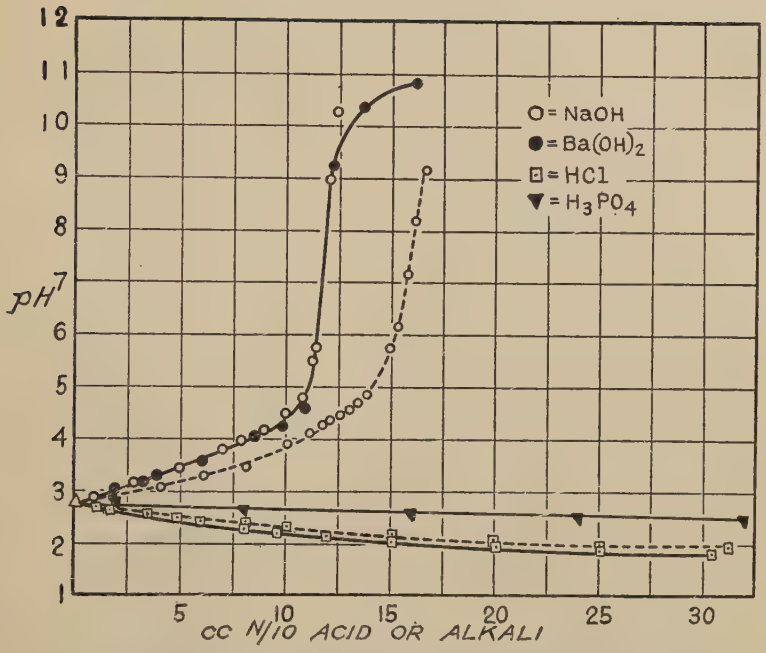
* The Leeds and Northrup portable potentiometer No. 7655 was employed throughout the work.

The titrations were run at room temperature and the pH value calculated with the temperature corrections as given in Clark[16] p. 457. The calomel electrode contained saturated potassium chloride solution and was the Hildebrand type as described and modified by Wilson and Kern.[17] The standard values for calomel electrodes were taken from Clark p 456.



PECTIN – 1 GRAM IN 100 CC. OF SOL
APPLE PECTIN—STRAIGHT LINE. CITRUS PECTIN—DOTTED LINE.

FIGURE 1.

abrupt as in the case of the apple pectin. More tenth normal alkali are required as the equivalent of one gram of citrus pectin than of the apple pectin.

TABLE II.

Titrations of purified apple pectin* with tenth normal alkali and acid.

	0.1 N acid or alkali	Observed E.M.F.	pH	0.1 N. acid or alkali	Observed E.M.F.	pH
$Ba(OH)_2$	0	0.4062	2.70	8.53	0.4806	3.97
	2.03	0.4217	2.97	9.34	0.4876	4.08
	3.25	0.4302	3.11	10.15	0.4952	4.21
	4.14	0.4370	3.23	10.70	0.5064	4.41
	4.47	0.4411	3.30	10.95	0.5138	4.53
	6.09	0.4521	3.49	12.30	0.7880	9.17
	7.11	0.4612	3.64	13.80	0.8525	10.27
	8.12	0.4690	3.77	16.24	0.8806	10.74

*The pectin solution contained one gram dry weight in 100 cc. of solution.

	0.1 N acid or alkali	Observed E.M.F.	pH	0.1 N. acid or alkali	Observed E.M.F.	pH
NaOH	0	0.4042	2.67	8.45	0.4810	3.98
	1.01	0.4135	2.83	9.05	0.4910	4.15
	2.10	0.4201	2.93	10.06	0.5082	4.44
	3.06	0.4305	3.12	10.86	0.5303	4.82
	4.02	0.4368	3.22	11.27	0.5661	5.43
	5.03	0.4483	3.41	11.46	0.5817	5.70
	6.07	0.4570	3.57	11.57	0.5825	5.71
	7.16	0.4688	3.77	12.07	0.7723	8.94
	8.05	0.4789	3.94	12.53	0.8470	10.21
HCl	0	0.4040	2.67	9.72	0.3750	2.17
	1.20	0.4022	2.64	12.05	0.3710	2.10
	2.49	0.3990	2.58	15.10	0.3651	2.00
	3.21	0.3953	2.52	20.08	0.3585	1.89
	4.02	0.3923	2.47	25.12	0.3542	1.84
	5.02	0.3890	2.41	30.52	0.3512	1.77
	6.02	0.3870	2.38	35.14	0.3485	1.72
	8.03	0.3810	2.27	40.16	0.3461	1.68
H_3PO_4	0	0.4060	2.70	24.00	0.3928	2.47
	2.00	0.4055	2.69	32.00	0.3896	2.42
	8.10	0.3998	2.59	40.00	0.3862	2.36
	16.00	0.3951	2.51	48.00	0.3828	2.30

TABLE III.

Titration of citrus pectin* with tenth normal acid and alkali.

NaOH	0	0.4029	2.65	12.88	0.5126	4.51
	0.80	0.4063	2.71	13.28	0.5185	4.61
	4.02	0.4225	2.98	13.68	0.5260	4.74
	6.04	0.4363	3.22	14.08	0.5360	4.91
	8.05	0.4533	3.40	14.49	0.5521	5.18
	10.06	0.4733	3.84	14.89	0.5832	5.71
	11.07	0.4873	4.08	15.29	0.6060	6.10
	11.67	0.4942	4.20	15.69	0.6650	7.10
	12.07	0.5005	4.30	16.10	0.7245	8.11
	12.47	0.5056	4.39	16.50	0.7830	9.10
HCl	0	0.4029	2.65	15.06	0.3758	2.19
	0.80	0.4008	2.61	20.08	0.3676	2.05
	1.61	0.3982	2.57	25.10	0.3618	1.95
	3.37	0.3933	2.49	30.12	0.3585	1.89
	5.02	0.3894	2.42	35.14	0.3550	1.83
	8.03	0.3845	2.34	40.16	0.3527	1.80
	1004	03817	2.29			

*The pectin solution contained one gram dry weight in 100 cc. of solution.

COMBINING WEIGHT OF PECTIN FROM PECTIN SALTS.

An attempt was made to establish the combining weight of pectin by means of its heavy metal salts. Fellenberg (1918) states that pectin is precipitated by copper sulphate, lead nitrate, the neutral and basic acetates of lead but is not precipitated by silver nitrate, cobalt nitrate, nickel sulphate, ferrous sulphate, zinc sulphate, manganese chloride, the chlorides of the alkaline earths nor by the alkaline salts.

Lead nitrate solutions varying in concentration from molar to tenth molar were used. The pectin solutions had been precipitated with alcohol three times and still contained about 3% ash. 500 cc. of the pectin solutions were stirred into 500 cc. of the solutions of lead nitrate. A gelatinous precipitate of lead pectate was obtained. This was stirred frequently for twenty-four hours, then filtered. The precipitates were washed repeatedly with 50% alcohol until no test for lead was obtained with sulphuric acid, then they were washed with 95% alcohol. The final drying took place in a vacuum oven at 50° C. to constant weight. The precipitates were analyzed for their lead content by converting the lead pectate into lead sulphate. The table of lead nitrate concentrations and the corresponding per cent. of lead in the precipitate is given below.

Conc. $Pb(NO_3)_2$ in moles	% lead in ppt.
1.00	17.96
0.75	18.11
0.50	17.56
0.25	16.96
0.10	15.22
0.075	14.98
0.050	13.98
0.025	13.10
0.010	12.24

It is seen that lead in the precipitates was not constant but increased with the concentration of the lead nitrate solutions.

Copper pectate was precipitated by stirring pectin solutions slowly into concentrations of copper chloride varying

from molar to tenth molar. The copper pectate was dried to constant weight as described in connection with the lead pectate series and ashed to cupric oxide. The copper content varied from 3.27% in the case of the tenth normal to 9.10% in case of the molar. The copper complex did not precipitate as completely from the more concentrated solutions of copper chloride as from the less concentrated solutions.

PECTIN SALTS PRECIPITATED WITH ALCOHOL AND WASHED WITH WATER.

To molar solutions of lead nitrate, ferric chloride and cupric chloride equal volumes of 1% pectin were added. Just enough nitric acid or hydrochloric acid were added respectively to dissolve the precipitates. Then equal volumes of 95% alcohol were added to each. Each precipitate was washed repeatedly with distilled water, then with 50% alcohol until the lead pectate washings gave no precipitate with potassium dichromate and the iron and copper pectate washings gave no coloration respectively with ammonium thiocyanate or potassium ferrocyanide. Below is tabulated some data obtained from the analyses of the dried precipitates.

Precipitate	% metal	gm. metal combined with 100 gm. pectin	gm. moles metal combined with 100 gm. pectin	gm. equiv. combined
Iron pectate	5.35	5.65	0.101	0.033
Lead pectate ...	11.06	12.44	0.060	0.030
Copper pecate ..	3.95	4.10	0.064	0.032

This is not sufficient data to conclude that there is chemical combination with the heavy metal salts but the gram equivalents of iron, lead and copper that combine with 100 grams of pectin are approximately equal.

THE ALKALI BINDING CAPACITY OF PECTIN.

What is meant by pectic acid is not clearly defined. The term[1] is applied to the water-insoluble pectic substances obtained by hydrolysis of pectin with the complete elimination of the methoxyl groups. Pectic acid may be variable in composition according to the type of hydrolysis; so there may be

a number of pectic acids, all methoxyl-free but retaining different amounts and kinds of non-methoxyl groups (i. e., arabinose, galactose, etc.).

Thus Carre and Haynes[5] saponified their pectin with sodium hydroxide, acidified with acetic acid and then precipitated pectic acid with calcium chloride. Wichmann and Chernoff [18] followed the same method of saponification, then boiled with 10% hydrochloric acid for five minutes to precipitate the pectic acid. They said that while boiling with the acid a furfural-forming radical split off and another fraction hitherto unknown which was precipitated as part of the pectate of Carre and Haynes. In the cyclopectic acid of Norris and Schryver,[8] calcium hydroxide was used to precipitate pectic acid. The gel after washing was allowed to stand in strong hydrochloric acid to convert the calcium pectate to pectic acid. They claimed that not only was methyl alcohol split off but also a hemicellulose like substance of variable and unstable composition. This was concluded since the furfural yield from the alkali filtrate did not appear to be constant.

Tutin[19] reported that purified pectin neutralizes in the cold and immediately "1.05% of its weight of sodium hydroxide." But if an excess of the alkali is kept in contact with pectin at room temperature, then an amount of sodium hydroxide equal to "12.35% of the weight of the pectin" is neutralized, the reaction being complete in a half hour.

In this work it was found that in titrating pectin with sodium hydroxide, equilibrium was quickly reached until after an equivalent quantity of alkali had been added. Then more than thirty minutes was required before a constant reading was obtained.

An attempt was made to find the alkali binding capacity of pectin as shown in Table IV. Varying amounts of sodium hydroxide were added to solutions of pectin of different concentrations and the pH values were determined as before. The hydroxyl ion values were calculated from the pH, taking the dissociation constant for water as 1×10^{-14}. The original normality (N) was known from the amount of alkali added;

the free normal sodium hydroxide (N′) was obtained from the curve given in Murray's work on Gum Arabic.[15] The data for that curve was obtained by preparing sodium hydroxide solutions of varying normalities in volumetric flasks and measuring the pH value. From the pH, the hydroxyl ion concentration was found as before. The difference between the original normality (N) and the free normality (N′) is a measure of the alkali combining with the pectin (n).

As noted from the last column of Table IV, the moles of sodium hydroxide neutralized by one gram of pectin vary from 0.00121 to 0.01602 or in a ratio varying from one to thirteen, the higher the pH the greater the neutralizing power. This would indicate that hydrolytic decomposition of pectin is readily caused by contact with appreciably alkaline solutions for a short time.

TABLE IV.

The Alkali Combining Capacity of Pectin.

Gm. pectin per liter	E.M.F.	pH	COH	Orig. N. NaOH (N)	Free N. NaOH (N′)	Combined N. NaOH (n)	Moles per gram of pectin
8.89	0.8470	10.198	6.34×10^{-4}	0.0112	0.0008	0.0104	0.00125
8.90	0.8368	10.060	6.34×10^{-4}	0.0115	0.0002	0.0113	0.00127
8.91	0.8450	10.165	1.46×10^{-4}	0.0110	0.0002	0.0108	0.00121
8.80	0.8703	10.595	3.94×10^{-4}	0.0121	0.0004	0.0117	0.00133
8.68	0.8738	10.653	$4.50\times10^{4-}$	0.0133	0.0005	0.0128	0.00147
8.51	0.8821	10.797	6.27×10^{-4}	0.0150	0.0008	0.0142	0.00166
13.31	0.9022	11.221	1.66×10^{-3}	0.0232	0.0020	0.0212	0.00159
12.82	0.9147	11.449	2.81×10^{-3}	0.0261	0.0030	0.0231	0.00180
15.15	0.8492	10.214	1.64×10^{-4}	0.0244	0.0002	0.0242	0.00159
14.92	0.8622	10.435	2.72×10^{-4}	0.0257	0.0003	0.0254	0.00170
14.60	0.8820	10.770	5.89×10^{-4}	0.0271	0.0007	0.0264	0.00180
14.28	0.8832	10.793	6.21×10^{-4}	0.0287	0.0008	0.0279	0.00181
10.00	1.0091	12.90	7.94×10^{-2}	0.2022	0.1375	0.0647	0.00647
10.00	1.0130	12.96	9.12×10^{-2}	0.3033	0.1598	0.1436	0.01436
10.00	1.0168	13.03	1.07×10^{-1}	0.3539	0.1937	0.1602	0.01602
10.00	0.9755	12.33	2.14×10^{-2}	0.0809	0.0277	0.0532	0.00532
10.00	0.9615	12.09	1.23×10^{-2}	0.0617	0.0166	0.0451	0.00451
10.00	0.9445	11.81	6.46×10^{-3}	0.0526	0.0084	0.0442	0.00442
9.52	0.9816	12.48	3.02×10^{-2}	0.0982	0.0427	0.0555	0.00571
2.50	0.9453	11.91	8.13×10^{-3}	0.0202	0.0108	0.0094	0.00376
2.50	0.9638	12.23	1.70×10^{-2}	0.0303	0.0228	0.0075	0.00300
2.50	0.9746	12.42	2.63×10^{-2}	0.0404	0.0355	0.0049	0.00192

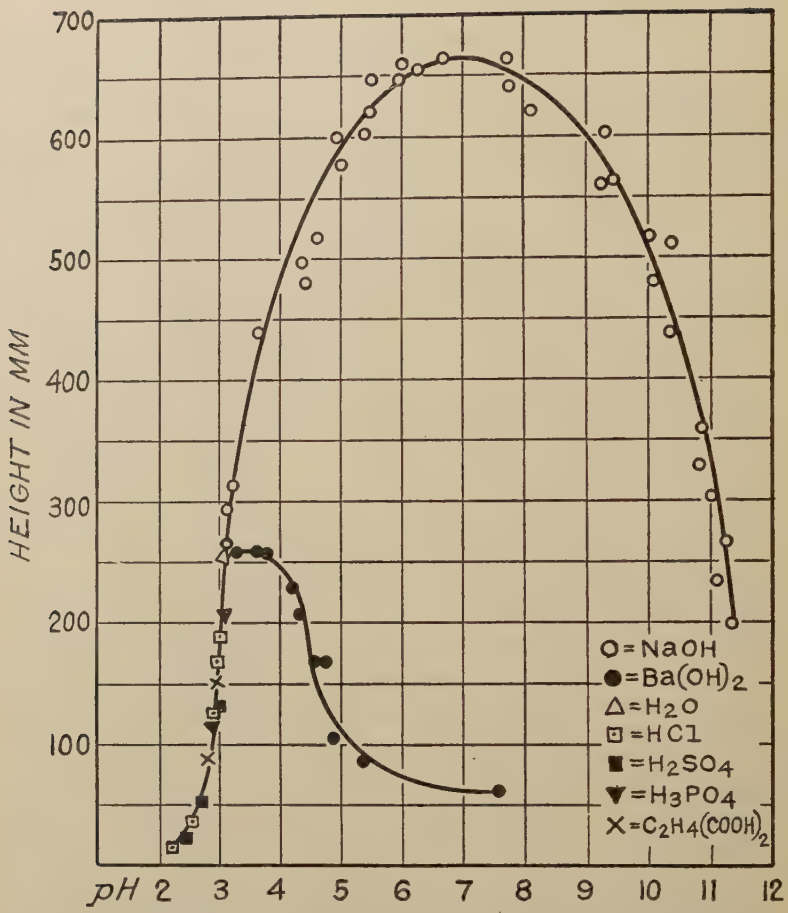
OSMOTIC PRESSURE AS A FUNCTION OF pH.

For these measurements pyroxylin bags permeable to acid or alkali but not to pectin served as membranes. The collodion used was "Merck U. S. P. X. containing 245 grains of ether per fluid ounce and 24% absolute alcohol." The test tubes, 3″ by 1″, were filled with the collodion and allowed to drain forty seconds, then they were air dried for about fifteen minutes, and then filled with water for ten minutes. The bags were always made up fresh for each set of osmotic experiments. Glass tubes, eight decimeters in length and having an average bore of 3.9 millimeters, served as manometers. The bags were closed with one-holed rubber stoppers, tied with twine, and filled through a small funnel with the pectin solution which contained one-half gram of pectin in 100 cc. of solution. Air bubbles were excluded and the funnel was replaced by a long glass tube. A coat of paraffin was painted around the twine as a safeguard against leaking. The bags were held in place in 400 cc. bottles by inserting the glass tube through two-holed rubber stoppers. The second hole was provided with a soda lime tube to exclude carbon dioxide. The top of the stopper capping the pyroxylin bag was adjusted level with the surface of the outside solution. The frame which held six bottles was then fixed in a thermostat regulated at 25° C.±0.01° and left for 24 hours, equilibrium being attained by that time.

The liquid rose in the tubes since the bags were permeable to the acid or alkali but not to the pectin. A small error was introduced due to the fact that as water entered the bags the pectin became diluted.

In Tables V and VI are recorded the heights of the liquid in the tubes measured in millimeters, and the pH of both inside and outside solutions. Figure II represents the same data. Varying concentrations of sodium hydroxide and barium hydroxide and of the acids, hydrochloric, sulphuric, succinic and phosphoric were used.

When water was used as the outside liquid, with no addition of acid or alkali, the pectin solution showed a pH value



OSMOTIC PRESSURE AS A FUNCTION OF pH

FIGURE 2.

of 3.11 and a rise of 257 millimeters. Addition of sodium hydroxide increased the rise to a height of 675 millimeters at pH 7.70, then greater alkalinity gave a gradual decrease. The outside solution was always the more alkaline. Results at higher pH could not be determined because the sodium hydroxide disintegrated the pyroxylin. The curve is somewhat flattened at the top between pH 6 and pH 8. Barium hydrox-

ide increased the rise but very slightly; after pH 4 further concentration of the hydroxide gave a depression. As some precipitation of barium pectate occurred at about pH 5 and increased at higher pH value, it was not attempted to make measurements beyond a pH of 7.65.

Addition of any acid resulted in a drop and regardless of what the acid was, the points all fall on the same curve. At a height of about 22 millimeters (measurement here being difficult) the acidity outside and inside was the same and remained the same upon the addition of more acid. The height then of 22 millimeters was due to the osmotic pressure of the pectin.

These osmotic pressure measurements show then that pectin, like arabic acid, combines with alkali but does not combine with acid—that is pectin is not amphoteric like protein.

TABLE V.

Osmotic Pressure as a Function of pH.

Acid or alkali	Outside soln. E.M.F.	pH	Inside soln. E.M.F.	pH	Observed O.P. in mm. soln.
H_2O	0.5155	4.56	0.4299	3.11	257
NaOH	0.5227	4.69	0.4305	3.12	265
	0.5485	5.13	0.4347	3.19	295
	0.5750	5.59	0.4388	3.26	314
	0.7245	8.11	0.4625	3.66	442
	0.7350	8.37	0.5056	4.42	483
	0.7393	8.37	0.5088	4.45	485
	0.7312	8.41	0.5041	4.36	499
	0.8060	9.48	0.5180	4.60	521
	0.8275	9.86	0.5197	4.73	521
	0.8546	10.30	0.5515	5.06	576
	0.8571	10.39	0.5380	4.94	602
	0.8702	10.60	0.5659	5.41	623
	0.8710	10.58	0.5696	5.48	648
	0.8750	10.64	0.5956	5.93	650
	0.8826	10.76	0.6159	6.27	657
	0.8857	10.85	0.6017	6.02	663
	0.8875	10.59	0.6400	6.67	665
	0.8896	10.92	0.7010	7.70	675
	0.8985	11.17	0.7234	8.11	627

Acid or alkali	Outside soln. E.M.F.	pH	Inside soln. E.M.F.	pH	Observed O.P. in mm. soln.
	0.9030	11.14	0.7942	9.29	603
	0.9032	11.26	0.7950	9.43	563
	0.9194	11.42	0.8435	10.06	518
	0.9096	11.22	0.8373	10.04	479
	0.9292	11.70	0.8796	10.90	358
	0.9252	11.49	0.8823	10.83	329
	0.9331	11.70	0.8910	11.04	235
	0.9360	11.80	0.9071	11.32	197
$Ba(OH)_2$	0.6300	6.50	0.4432	3.33	256
	0.6540	6.91	0.4497	3.44	261
	0.7304	8.23	0.4640	3.69	259
	0.7352	8.32	0.4695	3.78	257
	0.7450	8.52	0.4971	4.25	229
	0.7481	8.53	0.5010	4.32	205
	0.7500	8.54	0.5040	4.36	214
	0.8320	10.02	0.5136	4.53	165
	0.8306	10.00	0.5175	4.60	168
	0.8318	9.95	0.5251	4.73	168
	0.8360	10.09	0.5358	4.91	105
	0.8479	10.20	0.5650	5.39	85
	0.8535	10.29	0.6986	7.65	62

TABLE VI.

Osmotic Pressure as a Function of pH.

Acid or alkali	Outside soln. E.M.F.	pH	Inside soln. E.M.F.	pH	Observed O.P. in mm. soln.
HCl	0.4794	3.95	0.4285	3.18	190
	0.4640	3.69	0.4234	3.00	170
	4.4500	3.45	0.4215	2.96	125
	0.4475	3.41	0.4185	2.91	113
	0.4362	3.22	0.4157	2.87	101
	0.4331	3.16	0.4130	2.81	77
	0.4236	3.00	0.4104	2.27	67
	0.4187	2.92	0.4087	2.75	49
	0.4172	2.89	0.4050	2.68	47
	0.4115	2.79	0.4010	2.61	34
	0.4080	2.73	0.4010	2.61	35
	0.4005	2.61	0.3961	2.53	29
	0.4006	2.61	0.3988	2.58	31
	0.3976	2.55	0.3948	2.68	28
	0.3883	2.40	0.3883	2.40	23
	0.3838	2.30	0.3832	2.30	22

Acid or alkali	Outside soln. E.M.F.	pH	Inside soln. E.M.F.	pH	Observed O.P. in mm. soln.
H_2SO_4	0.4971	4.25	0.4280	3.07	190
	0.4632	3.67	0.4253	3.03	179
	0.4601	3.62	0.4221	2.97	121
	0.4402	3.28	0.4183	2.91	110
	0.4250	3.02	0.4144	2.84	62
	0.4195	2.93	0.4186	2.75	52
	0.4132	2.82	0.4060	2.70	42
	0.4024	2.64	0.4001	2.60	34
	0.3976	2.55	0.3962	2.53	25
	0.3971	2.55	0.3960	2.53	23
Succinic acid	0.5075	4.43	0.4255	3.03	243
	0.4561	3.55	0.4245	3.01	162
	0.4500	3.45	0.4195	2.93	148
	0.4435	3.34	0.4195	2.93	120
	0.4323	3.17	0.4160	2.87	88
	0.4264	3.05	0.4147	2.85	69
Phosphoric acid	0.4936	4.20	0.4293	3.10	208
	0.4721	3.83	0.4276	3.07	172
	0.4545	3.53	0.4226	2.98	139
	0.4424	3.32	0.4202	2.93	103
	0.4316	3.14	0.4171	2.89	81
	0.4278	3.07	0.4149	2.85	62
	0.4188	2.92	0.4086	2.74	50
	0.4132	2.82	0.4061	2.70	41
	0.4104	2.77	0.4044	2.67	37
	0.4128	2.64	0.4001	2.60	29

The observed osmotic pressure is the difference between the true osmotic pressure and the counter osmotic pressure. Suppose sodium hydroxide had been added to the pectin to the extent that the outside solution is alkaline and the inside solution is still acid, then on the inside are sodium, hydrogen and pectate ions and undissociated pectic acid. Outside are sodium and hydroxyl ions, the concentration of which can be determined from the pH of that solution.

Inside	Outside
$Na^+ R^-$	$Na^+ OH^-$
$H^+ R^-$	
HR	

The hydrogen ion concentration of the inside solution can also be measured directly. The measure of sodium ions can be found from the titration curve—that is by reading the amount of tenth normal sodium hydroxide required to bring the pectin to the pH of the inside solution. This is converted into gram moles contained in the inside solution, the total volume being known since the volume of the bag was measnred approximately and the volume in the tube found from the height of the liquid and the diameter of the tube. This concentration of gram moles of sodium ions in a definite volume can then be expressed in moles per liter.

Thus the osmotic pressure would be the sum of the sodium and the hydroxyl ions of the outside solution subtracted from the sum of the sodium and hydrogen ions and the pectin inside. The theoretical osmotic pressure of a gram molecular solution at 25° C. in terms of millimeters pressure of a column of water is

$$22.4 \times 760 \times 13.6 \times 298/273 = 2.5 \times 10^5 \text{mm.}$$

While the calculated osmotic pressures prove unsatisfactory quantitatively, in a qualitative way they support the above view of the chemical nature of pectin.

VISCOSITY AS A FUNCTION OF pH.

It is pointed out by Loeb [20] in his work on proteins that electrolytes have a similar effect on osmotic pressure, viscosity of protein solutions, and the swelling of protein gels since all three properties depend on osmotic pressure. The viscosity is due to the swelling of solid aggregates of protein and this swelling results from osmotic pressure inside the aggregates.

A theory of swelling based on Donnan's membrane equilibrium was given by Proctor[21] in 1914—that the osmotic pressure due to a greater number of crystalloidal ions inside the gel than outside, forces water to enter the gel and causes the swelling.

According to Einstein's equation,[22] applicable in this concentration, the swelling of aggregates will increase the viscosity in that the viscosity of the solution depends upon the

fraction of the volume of a solution occupied by the solute. In his equation, η_o is the viscosity of the water at the temper-

$$\eta = \eta_o (1 + 2.5\varphi)$$

ature of the experiment, η is the viscosity of the solution, and φ is the fraction of the volume occupied by the solute in the volume of the solution.

Murray[15] found that the addition of sodium hydroxide to his ashless gum arabic had an effect similar to that of gelatin, viscosity first increasing with the formation of the highly ionized sodium arabate, then decreasing with excess electrolyte, but unlike gelatin the addition of hydrochloric acid did not increase these properties. That there was no combination of acid with gum arabic.

The same was found true with purified pectin. The viscosity was measured by means of the Ostwald viscometer with a time of out flow of water of 93.2 seconds at 25° C. A 0.25% pectin solution was used since even at that dilution the ratio of its time of flow to that of water was more than 3.5 times and they were recorded as relative viscosity compared to water. The solutions were allowed to reach constant temperature in a thermostat regulated at 25° C. ± 0.01°. The measurements were made with varying concentrations of hydrochloric acid and sodium hydroxide. Barium hydroxide measurements were not feasible due to the formation of a precipitate barium pectate. In Table VII and Figure 3 are recorded the data of the viscosity measurements, after the acid or alkali solutions of pectin were one hour and twenty-four hours old. The water solution of pectin gave a relative viscosity of 3.18 at a pH of 2.98. Addition of hydrochloric acid decreased the viscosity until pH 2 was reached, beyond which there was no further effect. But when sodium hydroxide was added viscosity increased to a maximum at about pH 4.7 beyond which it abruptly fell. Also the viscosity curves for one hour and for twenty-four coincide to about that point but in more alkaline solutions, the twenty-four hour curve drops more toward the acid side. Hydrolysis is not complete in the shorter time. Pectin solutions, unlike gelatin, do not undergo

marked changes in viscosity upon standing for twenty-four hours.

Applying the Donnan's equilibrium theory to explain the swelling of pectin and sodium hydroxide as given by Procter and Wilson,[23] the cause of the entrance of water into the pectin and therefore the increase in volume is due to osmosis. The opposing force which limits the swelling is the cohesive force between the pectin molecules and ions which form the framework within which water is occluded.

The pectin ions cannot diffuse and therefore exert no measureable osmotic pressure, but the diffusible sodium ions held by the electrostatic forces of attraction to the pectate ions do exert osmotic pressure. Since there will be a difference in concentration of diffusible ions on opposite sides of the framework, a Donnan equilibrium is established.

Inside	Outside
Na^+ Pc^-	Na^+ OH^-
z z	x x
Na^+ OH^-	
y y	

Let x = the concentration of sodium and hydroxyl ions of the other solutions, y be the concentration of sodium and hydroxyl ions inside and z be the concentration of sodium ions in combination with the pectin.

$$x^2 = y\,(y + z)$$

Expressing the osmotic force for absorption of water as e

$$e = 2y + z - 2x$$

By substituting from thermodynamic and osmotic equation

$$e = -2x + \sqrt{4x^2 + z^2}$$

As the concentration of the base increases, z must increase faster than x. In very dilute solution most of the alkali would combine with the pectin but z reaches a limit depending upon the original concentration of the pectin. Now z must either approach the limiting value, or diminish if the ionization of sodium pectate were repressed by excess of y. In either case:

$$x = \propto \qquad \sqrt{4x^2 + z^2} = \sqrt{4x^2} = 2x$$

$$x = \propto \qquad e = -2x + 2x = 0$$

As x increases from zero, e must increase to a maximum and then decrease, approaching zero, regardless as to whether the ionization of sodium pectate is much repressed or not.

The curve for pectin shows this increase of viscosity to a maximum and then a decrease.

TABLE VII.

Viscosity as a Function of pH (1 hour)

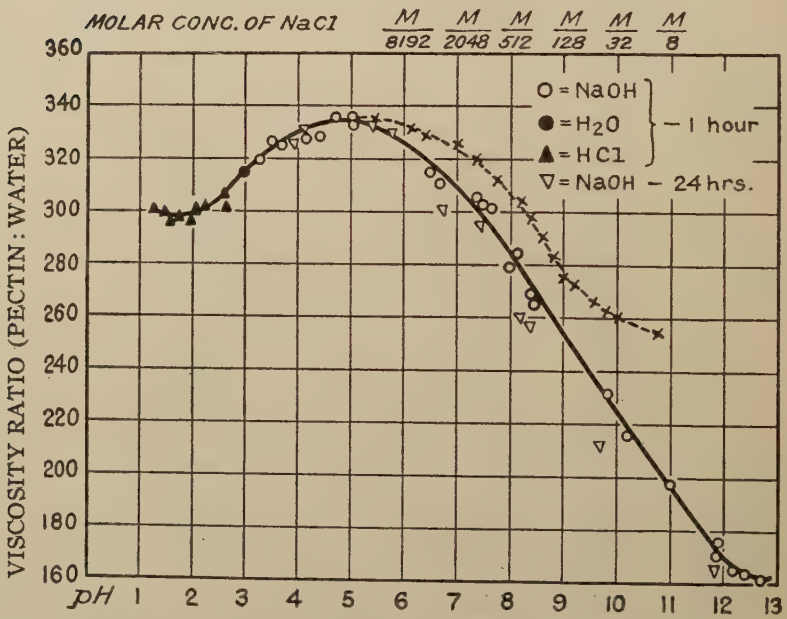
Acid or alkali	pH	Viscosity ratio	Acid or alkali	pH	Viscosity ratio
HCl	1.27	2.97	NaOH	5.08	3.28
	1.44	2.98		6.50	3.15
	1.56	2.96		6.69	3.10
	1.74	2.97		7.38	3.05
	1.99	2.96		7.46	3.03
	2.05	3.00		7.68	3.06
	2.23	3.01		7.97	2.79
	2.59	3.06		8.13	2.85
H_2O	2.98	3.18		8.40	2.69
				8.45	2.63
NaOH	3.30	3.19		9.88	2.32
	3.47	3.29		10.20	2.16
	3.73	3.25		11.03	1.98
	4.15	3.28		11.86	1.70
	4.69	3.36		12.76	1.62

Viscosity as a Function of pH (24 hours)

HCl	1.36	2.99	NaOH	5.01	3.26
	1.43	3.03		5.40	3.31
	1.74	2.96		5.76	3.30
	2.00	3.01		6.73	3.03
	2.61	3.08		7.45	2.83
				7.63	2.72
H_2O	3.08	3.19		8.21	2.59
				8.35	2.57
NaOH	3.31	3.28		9.69	2.11
	3.64	3.28		11.83	1.66
	3.90	3.25			
	4.10	3.31			
	4.44	3.28			

Increase of x and y by addition of a salt such as sodium chloride should also decrease e and likewise the viscosity. A pectin solution was brought to a pH of 5.49 and solutions con-

taining sodium chloride of concentrations ranging from zero to half molar were also brought to the same pH. From these stock solutions there were made up solutions containing 0.25% pectin and definite salt concentrations, the pH in each case being 5.49. The time of outflow through a viscometer was determined as before and the relative viscosity compared with water. Data is given in Table VIII and in Figure 3. The addition of sodium chloride causes only a drop, and no rise in the curve. The depressing effect on the viscosity of pectin is due to the same cause as the drop in the viscosity curve when too much alkali has been added, that is it is due to the sodium ions.



VISCOSITY AS A FUNCTION OF pH. - STRAIGHT LINE
" " " " NEUTRAL SALT SOLUTION - pH 5.49 - DOTTED LINE

FIGURE 3.

TABLE VIII.

Viscosity as a Function of Neutral Salt Concentration at the same Hydrogen Ion Concentration.

pH 5.49

Molarity		Viscosity ratio	Molarity		Viscosity ratio
M/6849	(0.0001)	3.33	M/157	(0.0064)	2.83
M/4310	(0.0002)	3.29	M/128	(0.0078)	2.75
M/2040	(0.0005)	3.26	M/103	(0.0097)	2.72
M/1279	(0.0008)	3.20	M/63.3	(0.0158)	2.65
M/603	(0.0016)	3.12	M/36	(0.0277)	2.62
M/512	(0.0020)	3.08	M/31.2	(0.0320)	2.60
M/373	(0.0027)	3.05	M/9.6	(0.1042)	2.53
M/293	(0.0034)	2.99	M/4	(0.2500)	2.56
M/250	(0.0040)	2.89			

EFFECT OF SUGAR ON OSMOTIC PRESSURE OF PECTIN SOLUTIONS

Series of osmotic cells were set up in an attempt to find the effect of sugar on the osmotic pressure of pectin solutions. Since the acidity of the pectin was found to invert sucrose, glucose was substituted. It forms a firm jelly with pectin just as sucrose does. Pyroxylin bags were prepared as for the previous osmotic experiments. To determine if the pyroxylin film was freely permeable to the sugar, preliminary experiments were made using sugar solutions of somewhat different concentrations outside and inside the bags. After two days the solutions outside and inside gave the same polariscope reading, thus proving that the membranes were permeable to glucose.

In the first series of osmotic cells, the 25 cc. bags were filled with solutions containing one gram of pectin and concentrations of glucose varying from 50 to 25 grams in 100 cc. of solution, while the outer solutions (about 200 cc.) contained glucose only and in the same concentration as the inner solutions. In the following table are given the polariscope readings of the same solutions forty-eight hours later and the height to which the liquid rose in the tubes.

POLARISCOPE READINGS* OF OUTSIDE SOLUTIONS

Original solution	48 hours later	Height in tube (cm.)
146.4	146.2	15.7
128.0	128.0	19.6
92.0	92.2	22.6
73.2	72.6	25.1
1% pectin solution inside, water outside		39.4

A second series was tried using pyroxylin bags of 10cc. capacity with 40 cc. of solution outside, since as the solutions approach the same volume, a change in concentration of the outer solution could be detected more easily.

POLARISCOPE READINGS* OF OUTSIDE SOLUTIONS

Original solution	48 hours later	Height in tube (cm.)
150.0	151.0	5.4
134.4	134.6	10.1
120.0	120.0	11.7
105.0	104.8	13.5
90.0	89.8	14.8
Water and pectin inside, water outside		32.5

It will be observed that the more concentrated the sugar solution, the lower the heights of liquid in the tube, indicating an association of pectin particles. The fact that there was no change in the polariscope readings of the outside solutions after forty-eight hours does not necessarily prove that sugar does not combine with the pectin since the amount of sugar that might so combine could be so small as to be out of the accuracy of this method. However within the limitations of this method, it is shown that sugar provokes an association of pectin particles when it jellifies in the presence of sugar.

* Ventzke Scale, using a 2 dcm. observation tube.

SUMMARY

1. The method of purification of the apple pectin has been described.

2. Pectin was found to combine stoichiometrically with the bases, sodium hydroxide and barium hydroxide, and the titration curves would indicate that pectin has properties of a strong acid.

3. Pectates precipitated by salts of iron, copper and lead show a metallic content of 0.03 to 0.033 equivalents per 100 gm. pectin.

4. Determination of alkali binding capacity of pectin is not possible due to the hydrolytic decomposition of pectin.

5. Osmotic pressure as a function of hydrogen ion concentration has shown that pectin unites with bases but not with acids.

6. The viscosity of pectin solutions rises to a maximum and then falls with increasing alkalinity.

7. Viscosity is decreased by the addition of a neutral salt.

8. Glucose added to pectin gives a decreased osmotic rise indicating an association of pectin particles when pectin jellifies in the presence of a sugar.

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